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Feasibility of Peripheral Blood Stem Cell (PBSC) and Peripheral Blood Mononuclear Cell (PBMNC) Separation in Children With a Body Weight Below 20 KG

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Nine children from 10 to 76 months (median 28.0), weighing 8.5 to 19.7 kg (median 13.0 kg) underwent peripheral blood stem cell separation (PBSCS) or peripheral blood mononuclear cell separation (PBMNCS), after insertion of a double-lumen central venous catheter (8–10 French). Separations were performed with a continuous flow blood separator (Fenwall CS 3000 plus), running a specially adopted separation-program. In 7 children (5 with neuroblastoma IV, 1 with multifocal Ewing's sarcoma, and 1 with rhabdomyosarcoma IV), stem cells were mobilized by application of G-CSF at a dosage of 15–27.7 μg/kg body weight (median 16.25) subcutaneously following high-dose chemotherapy, according to the disease-related protocols, whereas 2 children had PBMNCS to induce graft vs. leukemia (GvL)-reaction in the HLA-identical sibling suffering from relapsed chronic myelogenous leukemia (CML: n = 1), or chronic myelomonocytic leukemia (CMML: n = 1) after allogeneic BMT. In all cases, the collecting procedure was performed after filling the cell separator with priming solution consisting of 2 U of irradiated and washed packed red cells, 250 ml human albumin, and 0.9% NaCl. In the 7 patients with solid tumors between 0.45 and 62.7 × 10^6 CD-34 positive cells/kg body weight were separated; the patient who had the lowest yield was separated twice after another mobilizing course. Three patients (2 with neuroblastoma IV and 1 with multifocal Ewing's-sarcoma) underwent a double transplantation with 1–3 portions of the collected stem cells within a 5- to 6-week interval. Two children had a rapid engraftment on both peripheral blood stem cell transplantations (PBSCTs). The third child, who had the lowest yield and was separated twice had prompt engraftment at the first PBSCT but delayed and incomplete engraftment at the second PBSCT. One patient after adoptive immunotransfer with PBMNCs for relapsed CML is now 40 months in complete cytogenetic and molecular biological remission, whereas the other patient treated for relapsed CMML did not respond to the PBMNC-transfusion. The results indicate that PBSCS and PBMNCS can be performed in children with a body weight below 20 kg. Med. Pediatr. Oncol. 29:115–120, 1997.

Key words: PBSC/PBMNC separation; PBSC transplantation; children; body weight below 20 kg

INTRODUCTION

The poor prognosis of advanced childhood tumors may be improved by chemotherapy dose escalation followed by bone marrow stem cell rescue or peripheral blood stem cell transplantation (PBSCT) [1–4]. The advantage of PBSC is the more rapid restoration of bone marrow function after myeloablative therapy. PBSCs are a convenient source of progenitor cells, which can be easily mobilized and harvested without general anesthesia and without the discomfort of multiple BM aspirations [5,6]. PBSC can be mobilized by either chemotherapy or growth factors, or both [7–9]. The time to engraftment depends significantly on the amount of progenitor cells infused. Whereas in adults guidelines for optimized progenitor harvest have been developed few data are available in children [10–13]. Recently it has also been shown that separation of PBMNCs of the previous bone marrow donors can rescue a patient suffering from leukemic relapse due to GvL-reaction of the transfused PBMNCs [14]. In pediatric patients, leukapheresis is associated with various problems such as metabolic and hemodynamic disturbances, difficult venous access because of small vessels, and lack of compliance of the patients during the apheresis procedure. This study was designed to evaluate the feasibility of peripheral stem cell/PBMNC collection in small children with a body

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TABLE I. Patients' Characteristics*

Patient no.	Age (month)	Sex	Body weight (kg)	Diagnosis	Treatment protocol	Status at cytaphereses	Mobilizing chemotherapy	G-CSF	Interval between last course of chemotherapy and leukapheresis
1	29	f	12.4	Neuroblastoma IV	A-NB 94	CR1	CAV	16 μg/kg d1–d13	14
2	74	f	19.7	Ewing's sarcoma IV	EICESS 92	PR	VAVI	15 μg/kg d1–d7	8
3	15	f	10.5	Neuroblastoma IV	A-NB 94	CR1	CAV	15 μg/kg d1–d20	19
4	38	f	16.0	Neuroblastoma IV	A-NB 94	CR1	CAV	19 μg/kg d1–d18	17
5	10	f	8.5	Neuroblastoma IV	A-NB 94	CR1	CAV	19 μg/kg d1–d16	15
6	37	m	16.2	Neuroblastoma IV	A-NB 94	PR	CAV	27.7 μg/kg d1–d32	33
7	9	m	12.1	Rhabdomyosarcoma IV	CWS 91	PR	Carbo/VP16	16 μg/kg d1–d13	12

*CAV = Cyclophosphamide 70 mg/kg × 2, Adriamycin 25 mg/m^2 × 3, Vincristine 1 mg/m^2 × 3, Vincristine 1.5 mg/m^2 × 1; VAVI = Etoposide 150 mg/m^2 × 3, Adriamycin 20 mg/m^2 × 3, Vincristine 1.5 mg/m^2 × 1. Ifosphamide 2,000 mg/m^2 × 3; Carbo/VP16 = Carboplatin 150 mg/m^2 × 2, Vepesid 150 mg/m^2 × 2.

weight below 20 kg, and to determine the numbers of CD 34 positive cells or PBMNCs that can be harvested realistically.

PATIENTS AND METHODS

Patients or Probands

In the past 3 years 9 children (7 females and 2 males) with a body weight below 20 kg underwent PBSCS or PBMNCS. The median body weight was 13.0 kg (range 8.5–19.7 kg). The median age was 28.0 months (range 10–74 months). Five patients had neuroblastoma IV, 1 patient had multifocal Ewing's sarcoma, and 1 had rhabdomyosarcoma IV (Table I). In 2 children, unstimulated PBMNCs were collected and transfused to induce a GvL-reaction in the HLA-identical siblings suffering from relapsed CML (n = 1) or CMML (n = 1) after a previous allogeneic bone marrow transplantation. (Table II).

Chemotherapy Regimens and Growth Factors Prior to PSCS

In 7 patients with advanced tumor, progenitor cells were mobilized after a preceeding chemotherapy cycle according to the treatment protocol with G-CSF at a median dosage of 16.25 μg/kg/day (range 15–27.7 μg/kg/day). G-CSF was applied subcutaneously once daily for a median of 17 days (range 7–33 days). The interval between chemotherapy and the first separation procedure was median 13 days (range 8–30 days). Further details of chemotherapy and treatment protocols are listed in Table I.

Collection Procedure of PBSCs and PBMNCs and Storage of PBSCs

After finishing cytoreductive chemotherapy, CD 34 positive cells were examined daily in the peripheral blood, measured by FACS-analysis (FACS-Scan, Becton Dickinson, San Jose, CA) to determine the stem cell overshoot for optimal collection. Criteria for starting the procedure was the increase of CD 34 positive cells above 30/μl. PBSCs and PBMNCs were collected using a continuous flow blood cell separator (Fenwall CS 3000 plus) running the same specially adopted program. The volume of blood processed in the collections was twice the calculated circulating blood volume (circulating blood volume = 80 ml × kg body weight). The blood flow rates, calculated from the machine, ranged from 22–29 ml/minute (median 25 ml/minute). In all cases, 1,000 ml of priming solution consisted of 2 U of CMV-negative irradiated and washed packed red cells, 250 ml 20% human albumin, and 0.9% NaCL with a hematocrit of 30%. The priming volume utilized was about 300 ml. Acid-citrate dextrose (ACD-A) was used as anticoagulant in a ratio of 1:10 (return-phase). All children had central venous access using 8–10 French double-lumen catheters. The insertions were performed under ketamine anesthesia by the staff-pediatricians at the pediatric ward. Eight children had insertion of the central line via subclavian access, whereas one child had jugularis interna access. The children were given calcium gluconate 10% (100–200 mg/kg) perorally 1 hour after starting the collection. They did not get any sedation, but were entertained by their parents present in the collection room during leukapheresis. The autologous platelet-enriched plasma was automatically returned to the patient via inlet line. The collection products were automatically reduced to a vol-

TABLE II. PBMNC-Donor Characteristics

Proband no.	Age (month)	Sex	Body weight (kg)	Diagnosis of recipient	Number of PBMNCs /kg body weight/recipient
1	28	f	14.5	CML	4.97×10^8
2	16	f	9.8	CMML	2.61×10^8

ume of 50 ml by the separation machine and the total number of nucleated cells/kg body weight and the absolute number of CD 34 positive cells/kg body weight were calculated for each patient (Table III). After adding 5–10 ml of 20% dimethyl sulfoxide, 10 ml of 20% human albumin, and 30–35 ml of medium (IMDM, Gibco, Grand Island, NY), leukapheresis products were frozen using a controlled rate nitrogen freezer (Cryoson, Schöllkrippen, Germany) and stored in the liquid phase of liquid nitrogen without further manipulation. PBMNCs were also processed to a volume of 50 ml and transfused without further manipulation immediately after cytapheresis.

Transplantation Procedure

In three of 7 patients double PBSCT was performed. The conditioning regimen in the 2 patients with neuroblastoma IV consisted of etoposide 60 mg/kg/day × 1 and carboplatin 500 mg/m^2/day × 1 at the first PBSCT, and after 5 weeks in cyclophosphamide 45 mg/kg/day × 2, carboplatin 500 mg/m^2/day × 3, and melphalan 180 mg/m^2/day × 1 at the second PBSCT. The patient with multifocal Ewing's sarcoma was conditioned with melphalan 30 mg/m^2/day × 4 and etoposide 1,800 mg/m^2/day × 1 at the first PBSCT, and received etoposide 1,600 mg/m^2 and the same dose of melphalan after a 6.5-week interval (Table IV). The conditioning regimen was tolerated without major side effects by all patients. PBSCs were thawed in a 42°C waterbath and rapidly reinfused without any manipulation, 1–3 portions each at the first and second PBSCT. Mannitol diuresis was given in order to prevent DMSO toxicity. The reinfusions did not cause any side effects.

RESULTS

Leukapheresis

The median number of CD 34 positive cells was 10.34 × 10^6/kg body weight (range 0.45–62.7). (Table III/IV). A total of 42 collections were performed (range 4–8/patient, median 4/patient). The patient with the lowest yield of 0.45 × 10^6 CD 34 positive cells/kg had a second PBSCS after a second mobilization cycle with a harvest of 3.76 × 10^6 CD 34 positive cells. In the two cases with adoptive immunotransfer PBMNC number was 4.97 and 2.61 × 10^8/kg body weight of the recipient, respectively (Table II). The duration of the procedure was 80–180 minutes (median 112 minutes) with blood flow rates of median 25 ml/minute and a range from 22–29 ml/minute. A constant side effect was a decrease of platelets in peripheral blood counts (median decrease was 23%). The procedures were carried out without difficulties. No adverse side effects due to citrate toxicity were seen. The collection procedure was well tolerated by all patients.

Hematological Recovery

All children showed adequate three lineage engraftment, after reinfusion of collected PBSCs divided in different portions at the first PBSCT (Table IV). Total leucocyte counts exceeded 0.5 × 10^9/l with a median of 6 days and a range from 5–7 days. Platelet transfusion independence was reached by median 12 days with a range of 11–14 days (Table V). After the second PBSCT patient 1 had regular erythrocyte and leucocyte engraftment but a slightly delayed platelet recovery (platelet transfusion dependence up to day 48). Patient 2 showed prompt engraftment of all three lineages. Patient 3 who had the lowest CD 34 yield at the first PBSCS and had a second PBSCS (3.76 × 10^6 CD 34 positive cells/kg given in divided fractions on PBSCT 1 and 2) had delayed leucocyte recovery and remained erythrocyte and platelet transfusion dependent at PBSCT 2, and died on day 54 after the second PBSCT with persistent bone marrow involvement from active neuroblastoma metastases (Tables IV and V). Patient 1 and 2 have a complete remission of 26 and 24 months after the second PBSCT.

DISCUSSION

It is commonly accepted that the poor prognosis of advanced childhood tumors can be improved by chemotherapy dose escalation followed by bone marrow stem cell rescue or PBSCT. Adult studies suggest that PBSC autografts have some advantages over bone marrow grafts. Of major clinical relevance is the capacity to produce a more rapid engraftment. It has been reported previously that due to this fact, autologous PBSCT correlates with a lower incidence of febrile days, infections, shorter hospitalization, reduced antibiotic usage, and lower number of red cell and platelet transfusions as compared with conventional autologous BMT [15,16]. Despite these advantages, some potential problems may occur in children due to their low total blood volume, their small vessels, and their low body weight. Metabolic and hemodynamic disturbances, the difficulty of getting sufficient venous access providing sufficient blood flow

TABLE III. Results of Leukaphereses

Patient no.	Diagnosis	Number of NC × 10^8/kg body weight/Number of $CD34^+$ cells × 10^6/kg body weight 1	2	3	4	5	6	7	8	
1	Neuroblastoma IV	3.92/0.7	4.19/4.1	3.15/3.6	2.23/1.94					Total: 13.49/10.34
2	Ewing's sarcoma IV	1.84/3.0	7.0/21.0	6.6/24.0	5.2/14.7					Total: 20,64/62.7
3	Neuroblastoma IV	2,0/0.1	2.17/0.17	2.57/0.1	1.05/0.08					Intermediate: 7.79/0.45
						9.8/0.49	10.6/1.8	15.45/0.77	9.8/0.69	Total: 53.44/4.21
4	Neuroblastoma IV	1.34/4.33	1.16/1.71	1.8/6.44	2.5/6.2					Total: 6.8/18.68
5	Neuroblastoma IV	4.9/6.5	2.8/3.0	4.5/4.05	3.0/3.7					Total: 15.2/17.25
6	Neuroblastoma IV	0.31/0.81	0.88/1.42	0.78/1.34	0.75/1.65					Total: 2.72/5.22
7	rel. Rhabdomyo-sarcoma	2.65/2,12	2,7/1.38	7.56/4.0	5.1/2.14					Total: 18.31/9.64

TABLE IV. Conditioning Regimens in PBSCT (n = 3)

Patient no.	Diagnosis	Conditioning regimen 1	Conditioning regimen 2	Infused CD 34+ cells/kg Transplantation 1	Transplantation 2
1	Neuroblastoma	Etoposide 60 mg/kg Carboplatin 500 mg/m^2	Cyclophosphamide 45 mg/kg × 2 Carboplatin 500 mg/m^2 × 3 Melphalan 180 mg/m^2 × 1	0.7 × 10^6 (Portion 1)	7.7 × 10^6 (Portion 2 + 3)
2	Multifocal Ewing's sarcoma	Melphalan 30 mg/m^2 × 4 Etoposide 1,800 mg/m^2	Melphalan 30 mg/m^2 × 4 Etoposide 1,600 mg/m^2	24.0 × 10^6 (Portion 3)[a]	35.7 × 10^6 (Portion 2 + 4)
3	Neuroblastoma	Etoposide 60 mg/kg Carboplatin 500 mg/m^2	Cyclophosphamide 45 mg/kg × 2 Carboplatin 500 mg/m^2 × 3 Melphalan 180 mg/m^2 × 1	0.77 × 10^6 (Portion 7)	2.99 × 10^6 (Portion 5, 6, 8)

[a]Portion 1 was thawed and used for t11;22 EWS-Fly 1-PCR diagnosis and proved to be free of tumor cells.

TABLE V. Hematological Recovery (n = 3)*

Patient no.	Diagnosis	Days to leucocytes > 0.5 × 10^9/l PBSCT 1	PBSCT 2	Days to platelets > 20 × 10^9/l PBSCT 1	PBSCT 2	Days to reticulocytes > 15 × 10^9/l PBSCT 1	PBSCT 2
1	Neuroblastoma	7	8	16	48	9	14
2	Multifocal Ewing's sarcoma	5	5	10	20	11	16
3	Neuroblastoma	7	37	14	TD	16	TD

*TD = transfusion dependent.

rates, and a lack of compliance of children during leukaphersis may become problems that limit the collection of PBSCs in small patients. With regard to metabolic problems, the most important complication is hypocalcemia arising from relatively high citrate concentrations necessary in the return line, which is less tolerated by children as compared to adults.

Only a few reports are available showing the feasibility of PBSCS followed by autologous reinfusion in small children. Takaue et al. showed in 18 children aged below 4 years that the collection procedure is feasible, although the use of an arterial catheter, high amounts of ACD-A (1:6.7; inlet), and continuous calcium gluconate infusions expanded the clinical risk [17]. Deméocq et al. published their experience in 20 children weighing less than 20 kg. They used a continuous flow blood separator (Cobe spectra) running a program that allowed relatively low ACD-A rates (1:14; inlet). They used various forms of venous access: central catheters with femoral catheter return, femoral catheter with peripheral venous return, and a combination of central and femoral catheter and return through peripheral veins [18].

In this study we investigated the feasibility of PBSC/PBMNC collections in young children with low body weight (below 20 kg), who needed either myeloablative high-dose chemotherapy followed by autologous PBSCT

as treatment for their advanced solid tumors or donated PBMNCs as source for adoptive immunotherapy. The aim was to overcome some basic problems outlined by other investigators such as the clinical risk of using arterial lines, difficulties in placing sufficient central venous lines causing relatively low blood flow rates with demand of higher amounts of ACD-A, and longer duration of the collection procedure.

All our children were inserted with a double-lumen central venous catheter (size 8–10 French) [19]. This allowed blood flow rates of 25 ml (median), reducing the anticoagulant ratio to ACD-A 1:10 and shortening the duration of the collection procedures without any loss of PBSCs. No sedation was necessary and prophylactic peroral application of calcium gluconate prevented side effects from hypocalcemia. Our experience indicates that by using adequate central lines and the appropriate collection procedure, PBSCS or PBMNCS in children is as safe and effective as in adults.

We estimated a minimum of 3.0×10^6 CD-34 positive cells for successful autografting even in a double transplantation setting. In 6 children we could obtain this number of cells by 4 cytaphereses, while the patient with the lowest yield (0.45×10^6 CD 34 positive cells/kg) needed another 4 cytaphereses after a second mobilization. In the three transplanted patients, patients 1 and 2 had a time interval to hematological restitution within the range of previous published data. However, in patient 3, although recovery phase PBSCS yielded enough CD-34 positive cells (3.76×10^6/kg) after a second stem cell collection, complete three lineage engraftment could not be observed at the second PBSCT. This finding is in contrast to other reports assuming a high predictive value of CD 34 positive cells on the leucocyte and platelet recovery [20]. One might speculate that due to long (>4 months) intensive chemotherapy treatment prior to PBSC or the persistent bone marrow involvement seen at postmortem examination, the percentage of pluripotential progenitors among the total number of CD 34 positive cells was lower than expected. This is supported by the fact that stem cell response to mobilization protocols depends on various factors such as: BM involvement, prior treatment schedules, and the individual response of BM-progenitors to chemotherapy.

Our data suggest that PBSC- or PBMNC-collection is a safe and feasible method harvesting a sufficient number of stem cells for autotransplantation or mononuclear cells for adoptive immunotransfer in small children. Metabolic and hemodynamic problems may be prevented by using central venous lines providing sufficient blood flow rates and, therefore, tolerable ACD-A ratios in the inlet line. Further investigations are needed to establish the optimal measurement of stem cell levels in the collection product (i.e., CD-34 assay, LTIC investigations) [21] especially in children pretreated with high-dose chemotherapy.

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